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Research Article

# DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF ZILEUTON IN BULK AND IN PHARMACEUTICAL DOSAGE FORM BY UV SPECTROSCOPY

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**ABSTRACT**

The aim of this work was to develop and validate a simple estimation method of Zileuton in bulk powder and tablet using UV spectrophotometric method. The method was developed using methanol as a solvent and absorbance was measured at 229nm. Beers law was obeyed in the concentration range of 1-10µg/ml. Calibration curve shows a linear relationship between the absorbance and concentration. The line equation y=0.1045x + 0.0189 with $r^2$ of 0.9994 was obtained. The method was validated as per ICH guidelines. Simple, accurate and cost efficient spectrophotometric method has been developed for the estimation of Zileuton in bulk powder and tablet dosage form.

**Keywords:** Zileuton; UV determination; Validation

## INTRODUCTION

Zileuton is an orally active 5-lipoxygenase inhibitor that is being investigated in the treatment of ulcerative colitis. It may also have a role in the treatment of asthma and allergic rhinitis. IUPAC name of the Zileuton is N-[1-benzo (b) thien-2-ylethyl]-N-hydroxyurea. It is official in USP [1]. It is listed in the Martindale the complete drug reference [2] and Merck index [3].

Zileuton is used for the prophylaxis and chronic treatment of asthma in adults and children 12 years of age and older [4]. Contraindications are active liver disease or resistant elevation in transaminase at least 3 times the history of allergic reactions to Zileuton or any of its inactive ingredients [5]. Zileuton is a minor substrate of CYP1A2, 2C8/9, 3A4, and a weak inhibitor of CYP 1A2. The drug has been shown to increase the serum concentration or effects of theophylline, propranolol and warfarin, although significant increase in prothrombin time is not obvious. It is advised that the doses of each medication be monitored and/or reduced accordingly [6].

Extensive literature survey revealed that the determination of Zileuton and its inactive N-dehydroxylated metabolite in plasma by HPLC [7] and LC-MS/MS method in plasma [8] were reported. However there is no evidence for the estimation of Zileuton by UV spectrophotometry in bulk and in tablet formulation. So, an attempt was made to develop simple, cost effective and accurate UV spectrophotometric method for the estimation of Zileuton in bulk and in tablet formulation and to validate the developed method.

## MATERIALS AND METHODS

### Materials

Zileuton raw material was procured from AUROBINDO PHARMA LTD., Hyderabad, India. Tablet formulation GRILUTO-CR (Cadila Healthcare Limited, Goa, India) containing Zileuton 600 mg was purchased from local pharmacy. All reagents and solvents used were analytical grade. Ultra-pure water was obtained from a water purification unit.

### Instrumentation

UV spectrophotometric method was performed on LABINDIA UV-3000$^+$ Double Beam UV-Visible Ratio-Recording Scanning Spectrophotometer with pair of 10 mm matched quartz cell.

### Selection of solvent

Different solvents such as distilled water, methanol, acetonitrile, chloroform, diethyl ether and acetone were tried for estimation of Zileuton in tablet dosage form. Maximum sensitivity was found with Methanol. Hence, Methanol was selected as a solvent for study.

### Preparation of standard stock solution

25 mg of Zileuton standard substance was weighed and transferred into 25 ml volumetric flask separately, dissolved in methanol and made up to the volume with methanol. This solution contains 1mg/ ml concentration.

### Selection of wavelength for estimation and stability studies

The standard stock solution was further diluted with distilled water to get the concentration of 10 µg/ml and the solution was scanned between 200 - 400 nm using distilled water as blank. From the spectra, λmax was found to be 229 nm and was selected as analytical wavelength. The stability was performed by measuring the solution at different time intervals. It was observed that Zileuton in distilled water was stable up to 4 hours at the selected wavelength.

### Preparation of calibration graph

Working standard solution was prepared by pipetting 5 ml of the standard stock solution into a 25 ml volumetric flask and made up to the volume with distilled water to get the concentration of 200 µg/ ml. 0.5 – 5 ml were transferred into a series of 100 ml volumetric flasks and made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 229 nm. The calibration curve was constructed by plotting concentration Vs absorbance. Zileuton was linear with the concentration range of 1 - 10 µg/ ml at 229 nm.

### Quantification of raw material

2.0 ml of working standard solution was taken into a series of six 100 ml volumetric flasks and the volume was made up to mark with distilled water. The absorbance of these solutions was measured at 229 nm. The amount of Zileuton present in the raw material was determined by using slope and intercept values from calibration graph.

**Assay of tablet formulation**

Ten tablets of formulation (GRILUTO-CR containing 600 mg of Zileuton) were weighed accurately and the average weight of each tablet was found. The tablets were ground to a fine powder. The tablet powder equivalent to 25 mg of Zileuton was weighed and transferred into 25 ml volumetric flask. Added about 20 ml of methanol to dissolve the substance and the solution was sonicated for 15 minutes. Then it was made up to the volume to 25 ml with methanol (1 mg/ ml) and centrifuged for 15 minutes. The supernatant liquid was filtered through Whatmann filter paper No. 41. From the clear solution, further dilutions were made by diluting 5 ml into 25 ml with distilled water. 2 ml was pipetted out into a series of six 100 ml volumetric flasks and made up to the mark with distilled water to get the concentration of 4 µg/ ml of Zileuton, theoretically. The absorbances of six replicates were measured and the amount was calculated by using regression equation. This procedure was repeated for six times.

**VALIDATION OF UV SPECTROPHOTOMETRIC METHOD**

**Linearity and range**

Ability of the method to elicit test results that are directly proportional to the concentration of analyte. The linearity was determined by analyzing 6 independent levels of calibration curve in the range of 1– 10 µg/ ml at 229 nm. A calibration curve was plotted between concentration and absorbance and correlation coefficient and regression line equation for Zileuton were determined.

**Precision**

Precision may be defined as the concordance of a series of measurement of the same quantity. Intra-day precision was determined by analyzing Zileuton at three different time points of the same day and inter-day precision was determined by analyzing Zileuton three different time points on different days and %RSD was calculated.

**Accuracy**

It means the concordance between it and true or most probable value. Accuracy was determined by performing recovery studies by spiking different concentrations of pure drug in the pre-analyzed powder for infusion sample within the analytical concentration range of the proposed method at three different set at level of 80%, 100% and 120%.The amount of Zileuton was calculated at each level and % recovery were computed.

**LOD and LOQ**

The LOD and LOQ were estimated from the set of 6 calibration curves used to determine method linearity.

LOD=3.3σ/S and LOQ=10σ/S

Where, σ = the standard deviation of y-intercepts of regression lines

S = the slope of the calibration curve

**Table 1: Optical characteristics of Zileuton**

| Parameters | Results |
|---|---|
| λ max (nm) | 229 |
| Beer's Law Limit (µg/ ml) | 1 – 10 |
| Sandell's sensitivity ($g/cm^2$/0.001 A.U) | 0.00957 |
| Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$) | $2.5138\times10^4$ |
| Correlation coefficient (r) | 0.9994 |
| Regression equation (y=mx+c) | Y = 0.1045x + 0.0189 |
| Slope(m) | 0.1045 |
| Intercept(c) | 0.0189 |
| LOD | 0.1890 µg/ml |
| LOQ | 0.5728 µg/ml |
| Standard error | 0.0003 |

**Table 2: Quantification of raw material**

| Drug | Amount found (µg/ml)* | Percentage Obtained* (%) | Mean (%) | SD | %RSD | SE |
|---|---|---|---|---|---|---|
| Zileuton | 3.9820 | 99.55 | 100.51 | 1.0728 | 1.0674 | 0.0298 |
| | 4.0570 | 101.42 | | | | |
| | 3.9676 | 99.19 | | | | |
| | 4.0187 | 100.47 | | | | |
| | 4.0793 | 101.98 | | | | |
| | 4.0187 | 100.47 | | | | |

*Mean of six observations

**Table 3: Quantification of formulation**

| Drug | Labelled amount (mg/tab) | Amount found (mg/tab) | Percentage Obtained* | % Mean | SD | % RSD | SE |
|---|---|---|---|---|---|---|---|
| Zileuton | 600.0 | 588.70 | 98.11 | 99.38 | 1.2778 | 1.2857 | 0.0355 |
| | 600.0 | 600.48 | 100.58 | | | | |
| | 600.0 | 588.61 | 98.10 | | | | |
| | 600.0 | 607.06 | 101.18 | | | | |
| | 600.0 | 596.54 | 99.42 | | | | |
| | 600.0 | 593.39 | 98.90 | | | | |

*Mean of six observation

**Table 4: Intraday and interday analysis of formulation**

| Drug | Condition | Labelled amount (mg/tab) | Amount found (mg/tab)* | Percentage Obtained* | Average (%) | S.D | % R.S.D | S.E |
|---|---|---|---|---|---|---|---|---|
| Zileuton | Intraday | 600 | 599.42 | 99.90 | 100.00 | 0.5910 | 0.5910 | 0.0657 |
| | | 600 | 603.77 | 100.63 | | | | |
| | | 600 | 596.74 | 99.46 | | | | |
| Zileuton | Interday | 600 | 603.44 | 100.57 | 100.37 | 1.0006 | 0.9970 | 0.1112 |
| | | 600 | 595.16 | 99.28 | | | | |
| | | 600 | 607.51 | 101.25 | | | | |

*Mean of three observations

**Table 5: Recovery Study**

| Drug | Level (%) | Drug added (µg/ml) | Drug recovered (µg/ml) | % Recovery ± S.D | %R.S.D |
|---|---|---|---|---|---|
| Zileuton | 80 | 3.2350 | 3.2459 | 100.33±0.4854 | 0.4839 |
| | 100 | 4.0532 | 4.0691 | 100.39±0.4631 | 0.4613 |
| | 120 | 4.8480 | 4.8574 | 100.19±0.2815 | 0.2809 |

*Mean of three observations

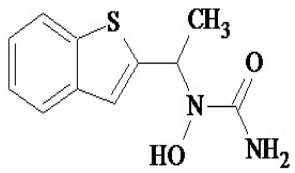


**Figure 1: Chemical structure of Zileuton**

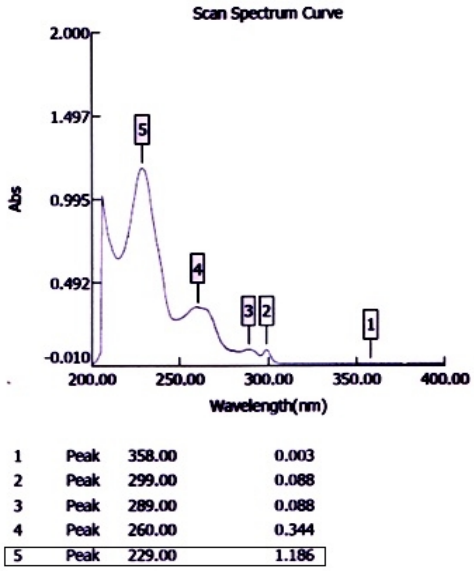


**Figure 2: UV spectrum of Zileuton**

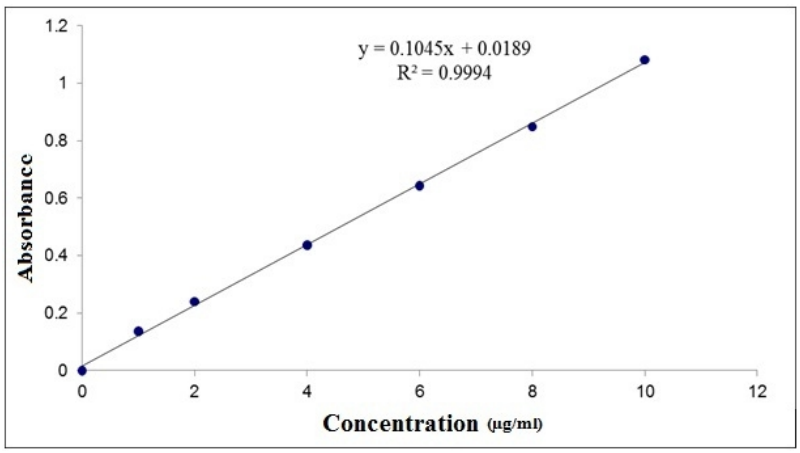


**Figure 3: Calibration curve of Zileuton**

## RESULTS

### Linearity

The linearity of Zileuton was found to be in the range of 1 - 10 µg/ ml. with correlation coefficient 0.9994. Calibration curve is shown in Fig.3. Optical characteristics were shown in Table 1.

### Precision

The percentage of Zileuton present in the prepared raw material solution was found to be 100.51% ± 1.0728 (Table 2). The percentage label claim present in the tablet formulation (GRILUTO-CR) was found to be 99.38% ± 1.2778 of Zileuton. The precision of the method was confirmed by the repeated analysis of formulation. The %RSD was found to be 1.2857 (Table 3).

Precision of the method was confirmed by intraday and inter day analysis. The percentage RSD value of the intraday and inter day analysis of Zileuton was found to be 0.5910% and 0.9970%, respectively (Table 4).

### Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. % Recovery for Zileuton was found to 100.19 - 100.39 (Table 5).

**Limit of detection and limit of quantification**

LOD and LOQ were found to be 0.1890 µg/ml and 0.5728 µg/ml, respectively.

## DISCUSSION

The proposed method for the determination of Zileuton in solid dosage form was found to be precise, selective, rapid and economical. Zileuton exhibited maximum absorption at 229 nm and obeyed Beer's law in the concentration range of 1 - 10 µg/ ml. The proposed method for the determination of Zileuton showed linear regression Y = 0.1045x + 0.0189 with correlation coefficient (R2) of 0.9994 (Figure 2). The percentage RSD for analysis of formulation was found to be within the limit.

Our studies revealed a recovery percentage of 100.19 - 100.39%, which indicates that the developed method was found to be accurate. The proposed methods can be used for the drug analysis in routine quality control and method proves to be more economical.

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## REFERENCES

1. The United States Pharmacopeia. The United States Pharmacopoeial Convention, Inc., Rock Ville, MD, USA, 3891-3893.
2. Martindale, The Complete Drug Reference, 34th edition, The Pharmaceutical Press, London-2005, 807.
3. The Merck Index, An Encyclopedia of Chemicals, drugs and biologicals, 14th Edition, Merck Research Laboratories, a division of Merck and Co. Inc., NJ, USA-2006, 10124.
4. http://www.drugbank.ca/drugs/DB00744
5. http://www.drugs.com/ppa/zileuton.html
6. http://www.en.wikipedia.org/wiki/zileutonl.html
7. Granneman GR, Braeckman RA and Erdman KA. Determination of a new 5-lipoxygenase inhibitor, Zileuton, and its inactive N-dehydroxylated metabolite in plasma by high performance liquid chromatography. Clinical Pharmacokinetics 1995; 29 suppl 2; 1-8.
8. Wiegand R., Wu J., Sha X., LoRusso P. and Li J. Simultaneous determination of ABT-888, a poly (ADP-ribose) polymerase inhibitor, and its metabolite in human plasma by liquid chromatography/tandem mass spectrometry. Journal of Chromatography B Analytical Technologies in the Biomedical and Life Sciences 2010; 878 suppl 3-4; 333-9.
9. Code Q2A, Text on Validation of Analytical Procedures. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland; 1994, 1 – 5.
10. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva Switzerland; 1996, 1 – 8.

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